Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Der Chemica Sinica, 2015, 6(6):72-77

Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Synthesis, characterization and antimicrobial evaluation of 5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives containing indole ring

Gollapalli Naga Raju*[1], Rudru Priyanka[2], Ari Manvitha Chowdary[1], Ponnekanti Sai Vaishnavi[1] and Rama Rao Nadendla[1]

[1]*Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Guntur, India*
[2]*Department of Pharmacy Practice, Chalapathi Institute of Pharmaceutical Sciences, Guntur, India*

ABSTRACT

Pyrazole is an important class of heterocyclic compound, has been shown to exhibit diverse biological and pharmacological activities such as anti-cancer, antioxidant, anti-inflammatory, antimicrobial, etc. In this study, a series of novel 5-(Thiophen-2-yl)-4,5-dihydro-1H-pyrazole derivatives containing indole ring have been synthesized. All the synthesized compounds have been characterized by using elemental analysis, FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopy and further supported by mass spectroscopy. Purity of all the compounds has been checked on thin layer chromatographic plate and HPLC technique. All the synthesized compounds were tested for their antibacterial and antifungal activity (MIC) in vitro by broth dilution method with two Gram-positive bacteria, two Gram-negative bacteria and two fungal strains. The biological activities of the synthesized compounds have been compared with standard drugs Ampicillin and Greseofulvin. The compounds exhibited significant antibacterial and moderate antifungal activities. These compounds can be further exploited to get the potent lead compounds. The detailed synthesis and the antimicrobial screening of the new compounds are reported.

Keywords: Pyrazole, Indole, Thiophene, Antibacterial activity, Antifungal activity.

INTRODUCTION

The synthesis of heterocyclic compounds has always drawn the attention of chemists over the years mainly because of their important biological properties. Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position and are also called as Azoles. Pyrazoles and its derivatives, a class of well known nitrogen heterocycles, occupy a prime position in medicinal chemistry for their diverse biological activities. They have been known to exhibit antimicrobial [1, 2], analgesic [3, 4], anticancer [5, 6], anti-tubercular [7, 8], anti-inflammatory [9, 10], antidepressant [11], anticonvulsant [12], hypoglycemic [13], antipyretic [14], antihelmintic [15], antioxidant [16] and herbicidal properties.

Considering the above observations and in connection to previous publications involving the synthesis of new biologically active heterocycles. Substitution of the heterocyclic moieties on the pyrazole ring is anticipated to have potential biological activity. In the present communication synthesis, characterization and the biological activity of various pyrazoles substituted with heterocyclic moieties are reported. Thus the efficient synthesis novel series of 5-(Thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives containing indole ring still represent highly pursued target.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. Formation of the compounds was checked by TLC on silica gel-G plates of 0.5 mm thickness and spots were located by iodine and UV light. All compounds were purified by recrystallization with suitable organic solvents. IR spectra were recorded on Brooker-ALPHA FT-IR instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. Purity of the synthesized compounds was checked by HPLC Agilent. The results are in agreements with the structures assigned. Elemental analysis of the all the synthesized compounds was carried out on Euro EA 3000 elemental analyzer and the results are in agreements with the structures assigned.

Synthesis of 1-Propyl-1*H*-indole-2-carbohydrazide: Methyl 1-propyl-1*H*-indole-2-carboxylate (2.17 g, 0.01 mol) in absolute ethanol (25 ml) was refluxed with hydrazine hydrate (1.0 ml) for 2 hour. After the completion of the reaction checked by TLC, the reaction mixture was cooled to room temperature. The separated solid was filtered, washed with cold ethanol and crystallized from ethanol.

Preparation of 3-Phenyl-1-(thiophen-2-yl)prop-2-en-1-one: These were prepared by condensation of 2-acetylthiophene and substituted aryl aldehyde in the presence of sodium hydroxide.

[3-(4-Fluorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl) methanone (1A): IR (KBr): 3481, 3108, 2923, 2871, 1697, 1590, 1578, 1554, 1492, 1135, 1028, 830 cm-1; MS: *m/z* = 391 [M+1]+; Anal. Calcd for $C_{22}H_{16}FN_3OS$: C, 67.85; H, 4.14; N, 10.79. Found: C, 66.88; H, 4.07; N, 10.57%.

[3-(3-Chlorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl) methanone (1B): IR (KBr): 3395, 3051, 2987, 2856, 1704, 1617, 1608, 1585, 1578, 1089, 1042, 740 cm^{-1}; MS: *m/z* = 406 $[M+1]^+$; Anal. Calcd for $C_{22}H_{16}ClN_3OS$: C, 65.10; H, 3.97; N, 10.35. Found: C, 64.87; H, 3.71; N, 10.23%.

1*H*-Indol-2-yl[3-(4-methylphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl] methanone (1C): IR (KBr): 3371, 3108, 3005, 2915, 1689, 1584, 1578, 1566, 1463, 1183, 1018, 870 cm^{-1}; MS: *m/z* = 385 $[M]^+$; Anal. Calcd for $C_{23}H_{19}N_3OS$: C, 71.66; H, 4.97; N, 10.90. Found: C, 70.49; H, 4.73; N, 10.56%.

1*H*-Indol-2-yl[3-phenyl-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]methanone (1D): IR (KBr): 3386, 3067, 2984, 2859, 1694, 1605, 1591, 1595, 1483, 1204, 1023, 845 cm^{-1}; MS: *m/z* = 371 $[M]^+$; Anal. Calcd for $C_{22}H_{17}N_3OS$: C, 71.14; H, 4.61; N, 11.31. Found: C, 70.66; H, 4.28; N, 11.07%.

[3-(4-Chlorophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl) methanone (1E): IR (KBr): 3365, 3048, 2988, 2851, 1682, 1571, 1580, 1536, 1456, 1134, 1093, 856 cm^{-1}; MS: *m/z* = 406 $[M+1]^+$; Anal. Calcd for $C_{22}H_{16}ClN_3OS$: C, 65.10; H, 3.97; N, 10.35. Found: C, 64.59; H, 3.61; N, 10.09%.

1*H*-Indol-2-yl[3-(2-nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl] methanone (1F): IR (KBr): 3486, 3152, 3046, 2846, 1676, 1651, 1612, 1551, 1491, 1008, 1281, 750 cm-1; MS: *m/z* = 416 [M]+; Anal. Calcd for $C_{22}H_{16}N_4O_3S$: C, 63.45; H, 3.87; N, 13.45. Found: C, 62.38; H, 3.53; N, 13.39%.

1*H*-Indol-2-yl[3-(4-nitrophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]methanone (1G): IR (KBr): 3386, 3089, 3018, 2914, 1689, 1586, 1579, 1523, 1469, 1087, 1028, 825 cm^{-1}; MS: *m/z* = 416 $[M]^+$; Anal. Calcd for $C_{22}H_{16}N_4O_3S$: C, 63.45; H, 3.87; N, 13.45. Found: C, 62.87; H, 3.59; N, 13.19%.

[3-(4-Aminophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl) methanone (1H): IR (KBr): 3519, 3076, 2964, 2852, 1704, 1609, 1581, 1568, 1542, 1159, 1065, 810 cm-1; MS: *m/z* = 386 [M]+; Anal. Calcd for $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50. Found: C, 67.78; H, 4.53; N, 14.28%.

[3-(4-Bromophenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl](1*H*-indol-2-yl) methanone (1I): IR (KBr): 3312, 3128, 3064, 2883, 1683, 1615, 1586, 1542 1453, 1286, 1003, 737 cm^{-1}; MS: *m/z* = 451 $[M+1]^+$; Anal. Calcd for $C_{22}H_{16}BrN_3OS$: C, 58.67; H, 3.58; N, 9.33. Found: C, 57.63; H, 3.27; N, 9.05%.

1*H*-Indol-2-yl[3-(4-methoxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl] methanone (1J): IR (KBr): 3428, 3162, 3029, 2918, 1711, 1610, 1591, 1542, 1508, 1162, 1063, 760 cm^{-1}; MS: *m/z* = 401 $[M]^+$; Anal. Calcd for $C_{23}H_{19}N_3O_2S$: C, 68.81; H, 4.77; N, 10.47. Found: C, 68.23; H, 4.23; N, 10.05%.

Scheme-1: 5-(Thiophen-2-yl)-4,5-dihydro-1H-pyrazole derivatives containing indole ring (1A – 1J)

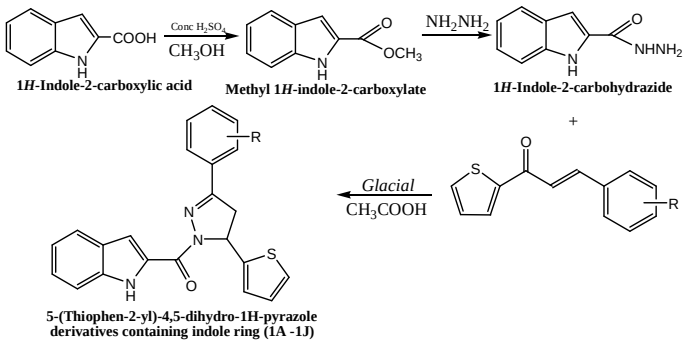
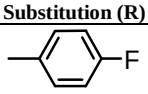
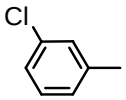
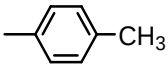
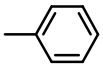
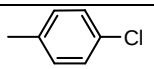
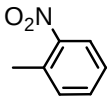
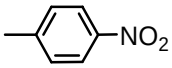
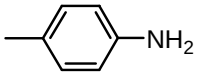
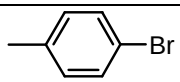
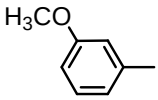


Table-1: Physical constant of synthesized 5-(Thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives containing indole ring

Compd	Substitution (R)	M.F	M.W	M.P (^{0}C)
1A		$C_{22}H_{16}FN_3OS$	389.44	135-136
1B		$C_{22}H_{16}ClN_3OS$	405.89	212-214
1C		$C_{23}H_{19}N_3OS$	385.48	187-189
1D		$C_{22}H_{17}N_3OS$	371.45	232-234
1E		$C_{22}H_{16}ClN_3OS$	405.89	221-223
1F		$C_{22}H_{16}N_4O_3S$	416.45	213-214
1G		$C_{22}H_{16}N_4O_3S$	416.45	188-189
1H		$C_{22}H_{18}N_4OS$	386.46	195-197
1I		$C_{22}H_{16}BrN_3OS$	450.35	173-175
1J		$C_{23}H_{19}N_3O_2S$	401.48	104-106

BIOLOGICAL EVALUATION:

Preparation of Culture Media

Nutrient broth was used as growth medium for bacteria and Saubouraud dextrose broth for fungi. Nutrient broth was prepared by dissolving 13gm of dehydrated powder (HI-media) in 100ml of distilled water. Saubouraud dextrose broth was prepared by dissolving 4gm of dextrose and 1gm of peptone in 100ml of distilled water. The media were sterilized by autoclaving at 15lbs pressure for 20 minutes.

Preparation of Stock Culture

Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100ml of sterile broth and incubated for 24 hours at 37^{0}C.

Standardization of Stock Culture

Stock cultures were placed in the incubator (37^0C for bacteria and 24^0C for fungi) and shaken well. One ml of stock cultures was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed with using a cyclomixer and serially diluted from 10^{-1} to 10^{-10}. From each dilution, 0.2ml was taken and spread on sterile nutrient agar plates for bacteria and Sabouraud dextrose agar plates for fungi, which were incubated for 18 hours. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum dilute tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15×10^8 cfu/ml.

Preparation of Working Stock Culture

Stock culture (0.1ml) was diluted with nutrient broth (100ml) and Sabouraud dextrose broth (100ml) respectively to obtain 10^5 cfu/ml. This was then used for further *in vitro* screening.

Preparation of Drug Dilutions

Solutions of the title compounds in DMSO (1mg/ml) were prepared and used for screening their antimicrobial activity.

Antimicrobial Screening:

Synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique. Test was carried out on four bacterial strains, namely *Staphylococcus aureus* (MTCC 96), *Staphylococcus pyogenus*, *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 443) and two fungal strains, namely *Candida albicans* (MTCC 227) and *Aspergilla niger* (MTCC 282).

Determination of MIC

The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in duplicate. To the first assay tube, 1.8ml of seeded broth and 0.2ml of title compound (1mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37^0C and 25^0C respectively for 24 hours for bacteria and fungi. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC).The MIC values of the test compounds are recorded in Table-2.

Table-2: Antimicrobial activity of 5-(Thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives containing indole ring

Compd	Minimal Inhibitory Concentration (µg/ml)					
	Antibacterial Activity				Antifungal activity	
	S.aureus	***S.pyogenus***	***E.coli***	***P.aeruginosa***	***C.albicans***	***A.niger***
1A	250	200	100	250	1000	250
1B	100	250	250	500	250	1000
1C	250	200	500	200	250	500
1D	250	500	250	100	1000	500
1E	500	100	200	125	250	200
1F	250	500	100	200	200	250
1G	100	100	500	250	250	500
1H	250	500	500	200	500	200
1I	100	250	200	500	1000	200
1J	500	200	100	250	200	1000
Ampicillin	250	100	100	100	NT	NT
Greseofulvin	NT	NT	NT	NT	500	100

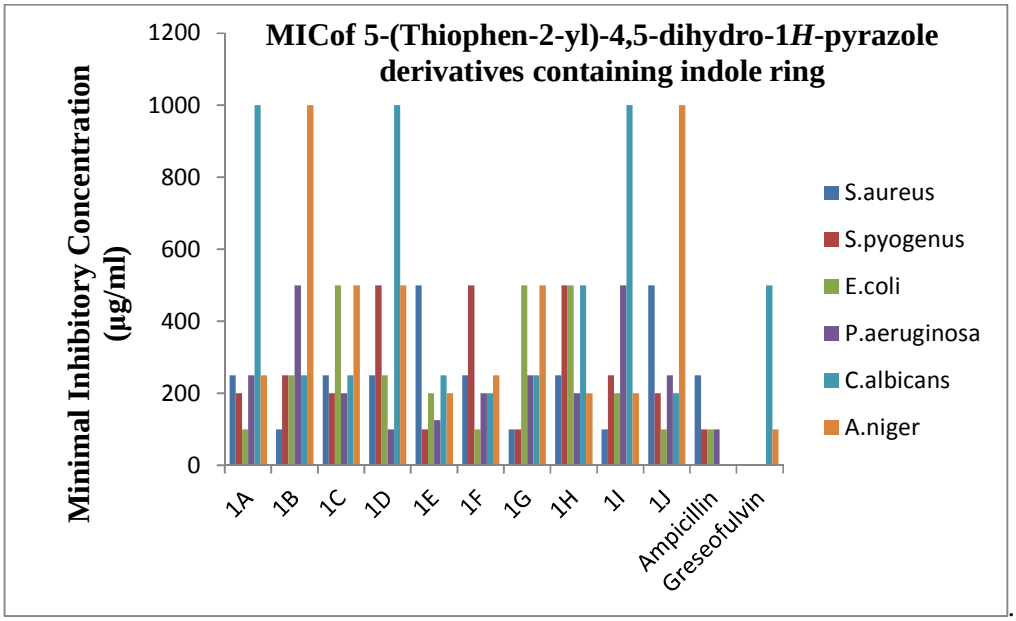


Figure 1: MIC of 5-(Thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole derivatives containing indole ring

RESULTS AND DISCUSSION

1*H*-Indole-2-carbohydrazide was dissolved in glacial acetic acid, added substituted chalcones and refluxed on an oil bath for 12 hours to prepare 5-(Thiophen-2-yl)-4,5-dihydro-1*h*-pyrazole derivatives containing indole ring (**1A to 1J**). All the synthesized compounds were subjected to antimicrobial screening by estimating the minimum inhibitory concentration (MIC) by adopting serial dilution technique.

The data recorded in Table 2 indicated that compounds **1B, 1G** and **1I** are more potent towards *Staphylococcus aureus.* The compounds **1A, 1C, 1D, 1F** and **1H** are moderately potent towards the *Staphylococcus aureus*. Compounds **1E** and **1G** are moderately potent towards the *Streptococcus pyogenes.* Compounds **1A, 1F** and **1J** were moderately potent towards the *Escherichia coli*. Compounds **1D** and **1E** are moderately potent towards the *Pseudomonas aeruginosa.* All these compounds are compared with the standard reference (Ampicillin) for their antibacterial activities. The compounds **1B, 1C, 1E, 1F, 1G** and **1J** are more potent towards the *Candida albicans*. All these compounds are compared with the standard reference (Greseofulvin) for their antifungal activities.

CONCLUSION

In this study, certain 5-(Thiophen-2-yl)-4,5-dihydro-1*h*-pyrazole derivatives containing indole ring were synthesized and evaluated for their antimicrobial activities. Results revealed that the compounds exhibited significant *in-vitro* antimicrobial activity. Compound **1A, 1B, 1G**, and **1J** are more potent. Remaining compounds also showed moderate to weak antimicrobial activities. The study would be a fruitful matrix for the development of 5-(Thiophen-2-yl)-4,5-dihydro-1*h*-pyrazole derivatives containing indole ring for further bio-evaluation.

Acknowledgement
The authors are grateful to Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Guntur for providing facilities to perform this research work.

REFERENCES

[1] P. Priyadarsinia, B. Ujwalaa, C. Venkata Raob, and V. Madhava Rao, *Der Pharmacia Lettre*, **2012**, 4 (4):1123-1128.
[2] K. Karrouchi, Y. Charkaoui, K. Benlafya, Y. Ramli, J. Taoufik, S. Radi and M. Ansar, *J. Chem. Pharm. Res*., **2013**, 5(3):1-6

[3] A.M. Vijesh, *European Journal of Medicinal Chemistry*, Volume 62, April **2013**, Pages 410–415.
[4] Alam MM, *Acta Pol Pharm*, **2013** May-Jun; 70(3):435-41.
[5] R. S. Gouhar, O. A. Fathalla and S. S. Abd El-Karim, *Der Pharma Chemica*, **2013**, 5 (6):225-233.
[6] George Mihai Nitulescu, Constantin Draghici and Octavian Tudorel Olaru, *Int J Mol Sci*. **2013** Nov; 14(11): 21805–21818.
[7] Pathak RB, *Bioorg Med Chem Lett*, **2012** Aug 1; 22(15):5129-33.
[8] Vimal I Patel and Dr.Bhavesh Patel, *International Journal of Pharma and Bio Sciences*, Vol.1, Issue- Oct-Dec.**2010**.
[9] Reshma J. Nevagi, *Der Pharmacia Lettre*, **2014**, 6 (5):274-284.
[10] Pawan Kumar, Navneet Chandak, Pawan Kaushik, Chetan Sharma, Dhirender Kaushik, Kamal R. Aneja & Pawan K. Sharma, *Medicinal Chemistry Research*, November **2012**, Volume 21, Issue 11, pp 3396-3405.
[11] Secci, *Current Medicinal Chemistry*; Nov **2011**, Vol. 18 Issue 33, p5114
[12] Prashant Aragade, Sadhana Kolhe, Hemant Kamble, Dwarkadas Baheti, Veeresh Maddi, *International Journal of Drug Design and Discovery*, Volume, 3 Issue 1 January – March **2012**, 688-693.
[13] Prasanna A. Datar and Sonali R. Jadhav, *International Journal of Medicinal Chemistry*, Volume **2015** (2015).
[14] Malvar Ddo C, *Life Sci*, **2014** Jan 30; 95(2):81-8.
[15] Balaji.P.N, D.Ranganayakulu, K.Ramya yadav, J.jayamma, S.Reddy kumar, C.Sivaramaiah*; Int.J. PharmTech Res*. **2014**,6 (7),pp 1970-1975.
[16] Khalid Karrouchi, Laila Chemlal , Latifa Doudach , Jamal Taoufik , Yahia Cherrah , Smaail Radi , My El Abbes Faouzi and M'hammed Ansa, *Journal of Pharmacy Research*, **2014**,8(8),1171-1177.